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SUPPLEMENTARY MATERIAL

corresponding to:

Real time dynamics of β-catenin expression during *Hydra* development, regeneration and Wnt signalling activation

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Regulatory *cis*-elements within the β-cat promoter sequence were predicted using the standard method of Position Specific Scoring Matrices, *Possum* (http://zlab.bu.edu/~mfrith/possum/), which detected several *cis*-elements, including the TCF binding site GTTTGAT, i.e. TCF-site3 core recently identified (Nakamura *et al*., 2011).

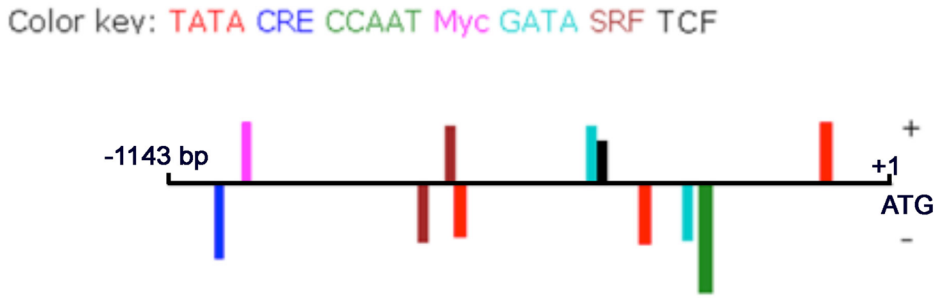
Transient transfection in *Hydra*

Functional assay of the recombinant β-cat-promoter upstream the eGFP reporter was performed by transient transfection, using a modified gene gun method.

Plasmid DNA was precipitated on gold bead and then delivered on *Hydra* tissues by gene gun (2 shots at 6 cm). Transfected polyps were allowed to recover at 18°C and monitored by fluorescence microscopy at different times post transfections. 96 h post transfection a clear fluorescent signals was detected on the hypostome, showing functionality of the recombinant reporter system (**Fig**. S2). High levels of β-cat expression in the hypostome were later detected also in the stable β-cat-eGFP transgenic polyps.

References

NAKAMURA, Y., TSIAIRIS, C.D., OZBEK, S. and HOLSTEIN, T.W. (2011). Autoregulatory and repressive inputs localize Hydra Wnt3 to the head organizer. *Proc Natl Acad Sci USA* 108: 9137-42.



motif	position	strand	sequence
CRE	-980	-	gtttacgtcaaa
Myc	-946	+	accatgctcc
SRF	-689	-	acctttgctgggg
SRF	-636	+	attcatgaatggc
TATA	-636	-	gtgttgtttttatat
GATA	-444	+	tacggataacaag
TCF	-432	+	gtttgat
TATA	-367	-	aaggtggttatatac
GATA	-304	-	acaactatcttgt
CAAT	-279	-	gatttgattggcgcct
TATA	-102	+	ttttatatgcgcgct

Fig. S1. *In silico* analysis of 1143 bp DNA fragment of β-cat promoter. *The Possum on line software was employed to characterize regulatory sequences contained within putative DNA promoter sequence. Consensus sequences are shown relative to the β-cat gene transcription starting site.*

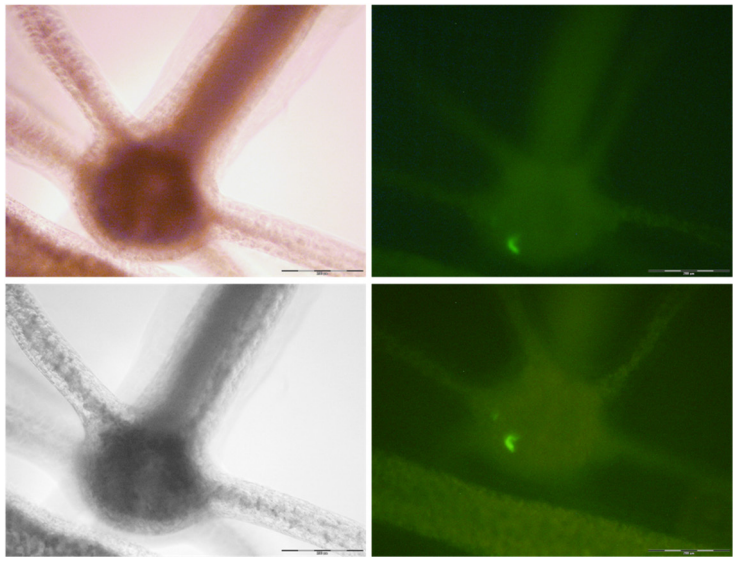


Fig. S2. *In vivo* imaging of β-cat-GFP transfected polyps. *Bright field (left) and fluorescence (right)* in vivo *images of two polyps 96 h post biolistic bombardment. The hypostome express the fluorescent reporter. Scale bars, 500 μm.*

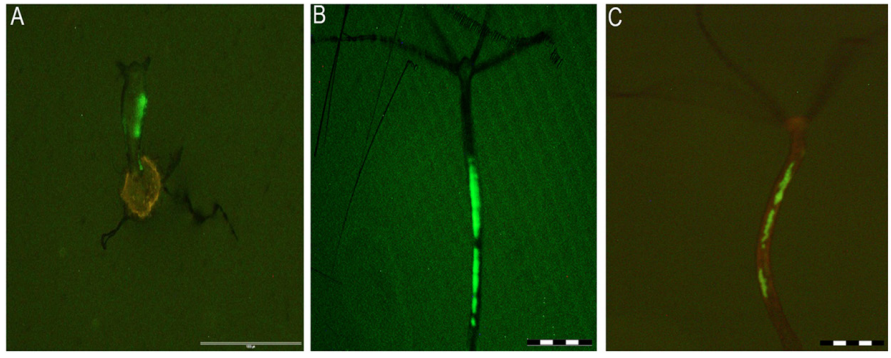


Fig. S3. Development of a β-cat reporter line from an embryo to a primary polyp. *Hatching of embryo injected with the β-cat-eGFP plasmid* **(A)**, *and mosaic polyps, showing patches of GFP positive cells* **(B, C).** *Scale bars, 200 μm.*

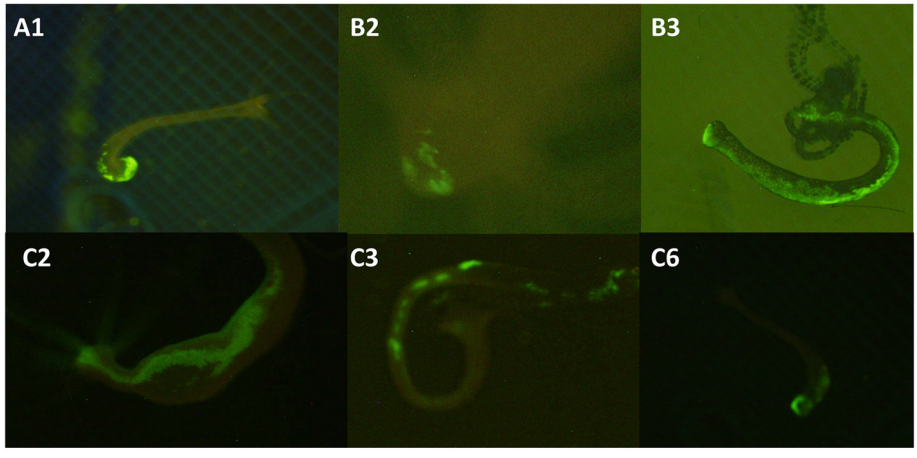


Fig. S4. Mosaic polyps hatched from injected embryos. *β-cat driven eGFP reporter gene expression is detected in different regions of the polyps. Clonal propagation from eah polyp generated ECT and ENDO lines, and in case of C3, C2, and B3, also INT lines.*

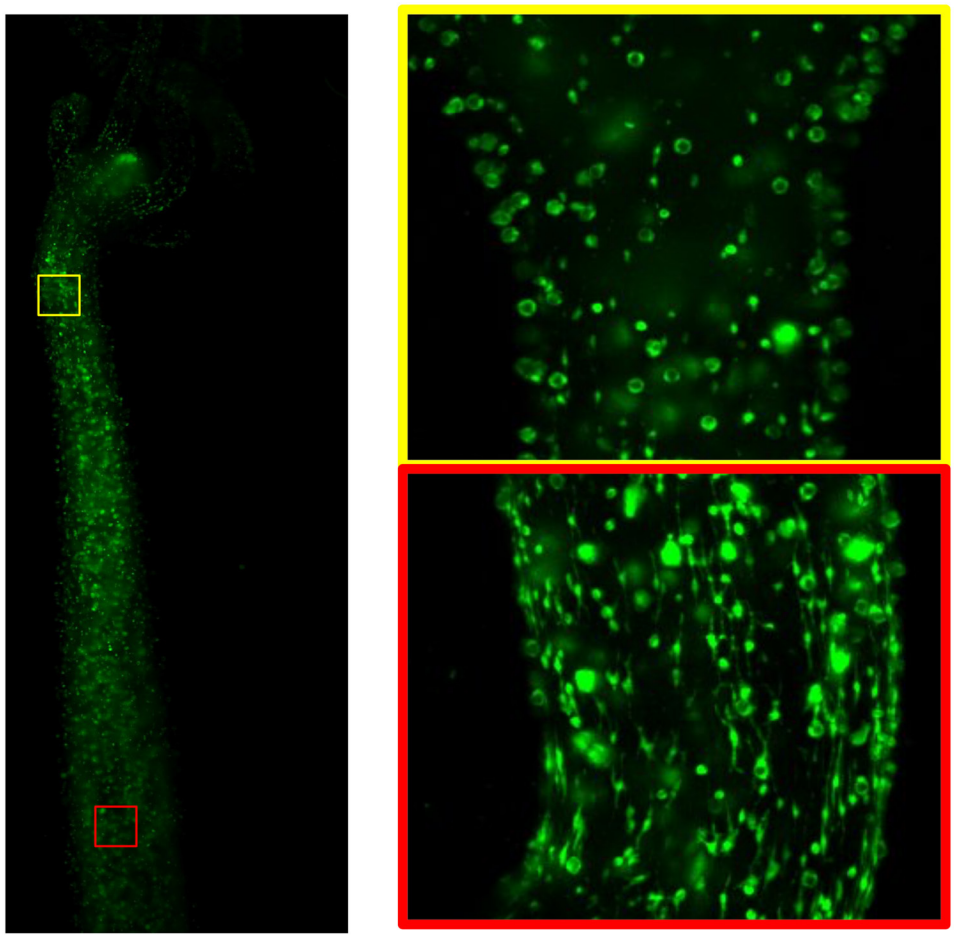


Fig. S5. *In vivo* light sheet microscopy imaging of β-cat-GFP INT *Hydra.* *Fluorescence appears as a punctuated pattern extending throughout the body length, from the foot to the tentacles tips. Details of Maximum Intensity Projection are shown in the yellow and red framed images, illustrating eGFP+ interstitial cells in the upper body and a neuron dense region with cells oriented parallel to the major polyp axis on the peduncle region.*

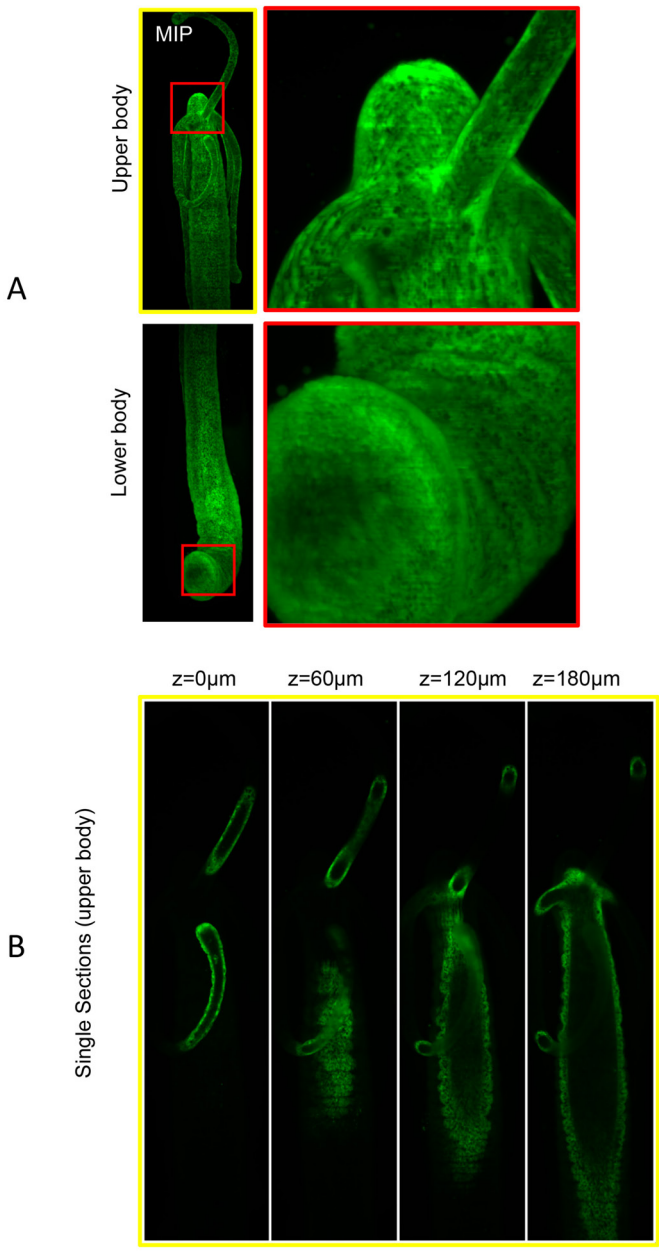


Fig. S6. Light sheet microscopy imaging of β-cat-GFP ECTO *Hydra*. *Maximum intensity projections of the upper and lower body regions* **(A),** *and details of the hypostome and foot (red squares). Single sagittal sections of the stack acquired at different depths, every 60µm* **(B)**. *eGFP+ cells are present into the ectodermal layer.*

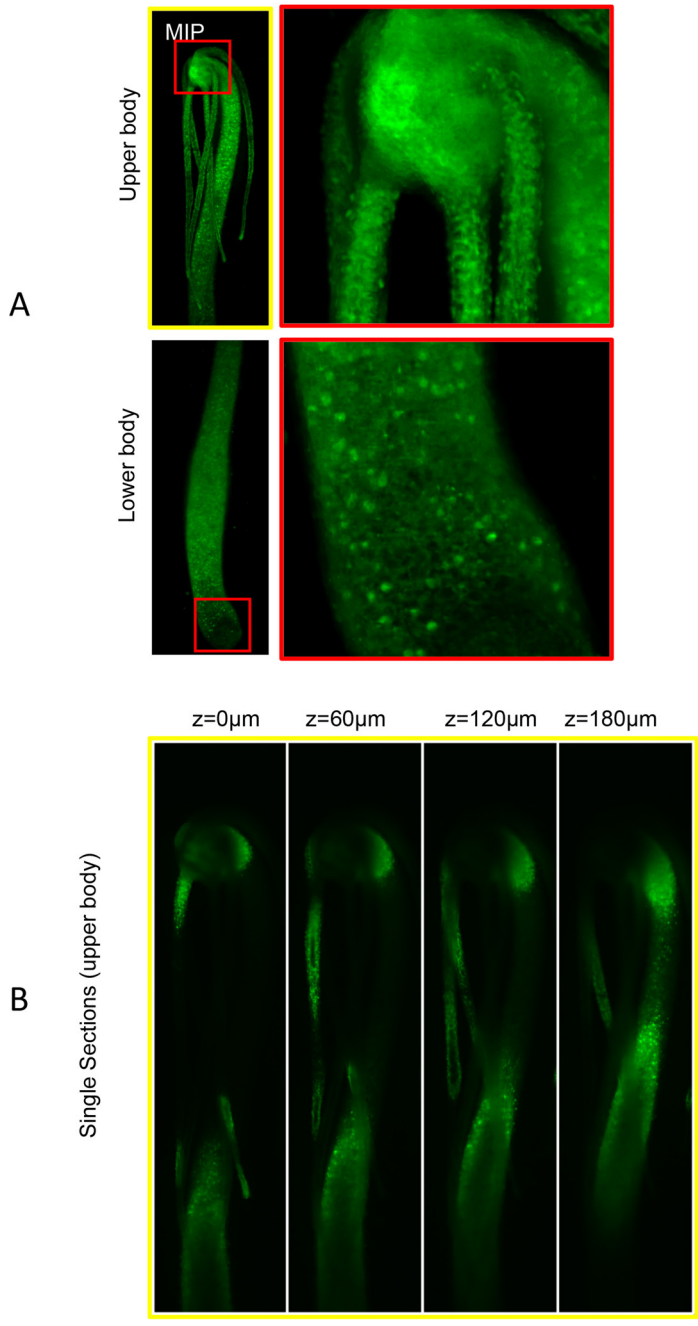


Fig. S7. *In vivo* light sheet microscopy imaging of β-cat-eGFP ENDO *Hydra*. *Maximum intensity projections of the upper and lower body regions* **(A),** *and details of the head and foot regions (red squares). Single sagittal sections of the stacks acquired at different depths, every 60µm* **(B).**

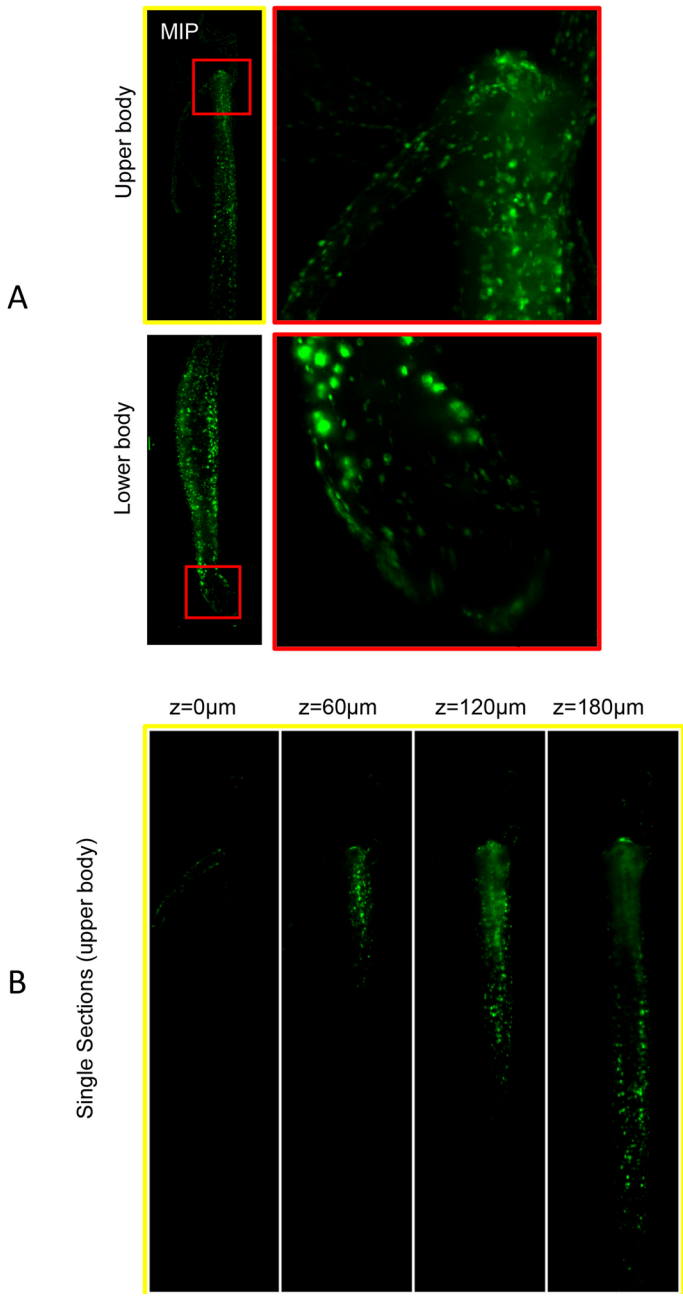


Fig. S8. *In vivo* light sheet microscopy imaging of β-cat-GFP INT *Hydra*. *Maximum intensity projection of the upper and lower body regions* **(A)**, *and details of the head and foot regions (red squares). Fluorescence appears as a punctuated pattern. Single sagittal sections of the stacks acquired at different depths, every 60µm* **(B)** *show the fluorescence localized in the endodermal layer as well, due to the gland cells where the β cat promoter is active.*

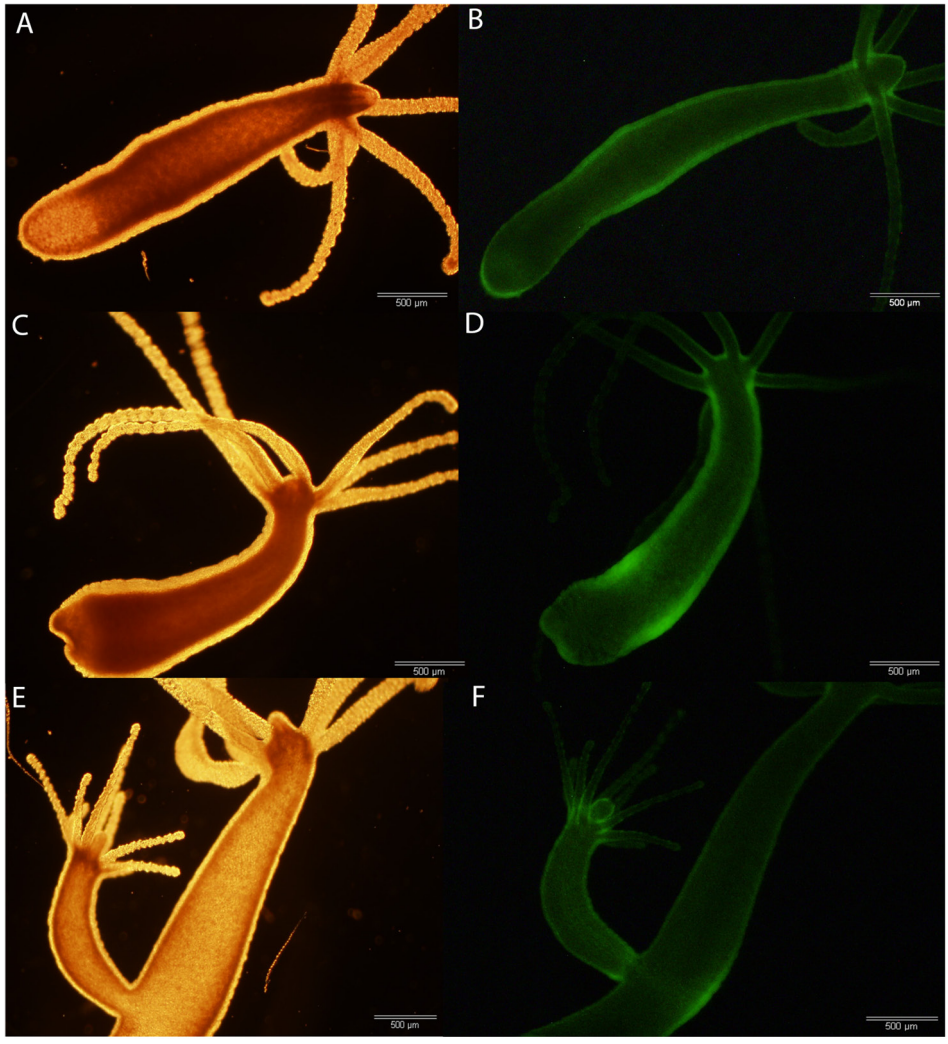


Fig. S9. β-cat reporter expression during budding of a β-cat-eGFP ECTO transgenic polyp. *Dark field* **(A)**, **(C)**, **(E)**, *and fluorescence* **(B)**, **(D)**, **(F)** in vivo *images show that approximately 24 h before budding the promoter is active in a ring like zone that appear increasingly fluorescent in the protrusion region, and in the hypostome of the new bud. Scale bars, 500 μm.*

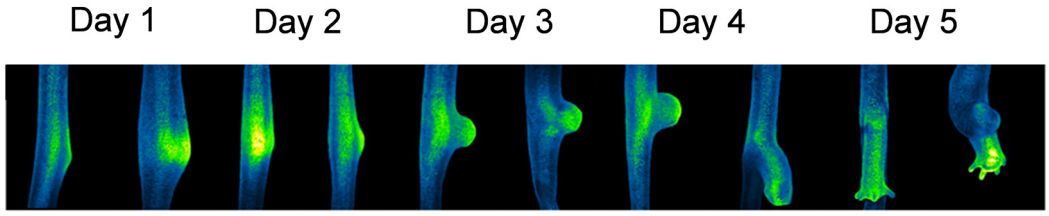


Fig. S10. Time-lapse acquisition by light sheet fluorescence microscopy of a β-cat eGFP ECTO transgenic Hydra during the budding process. *Images were acquired every 40 min. Representative images captured every 24 h are reported. Data are also shown in Fig. 2C.*

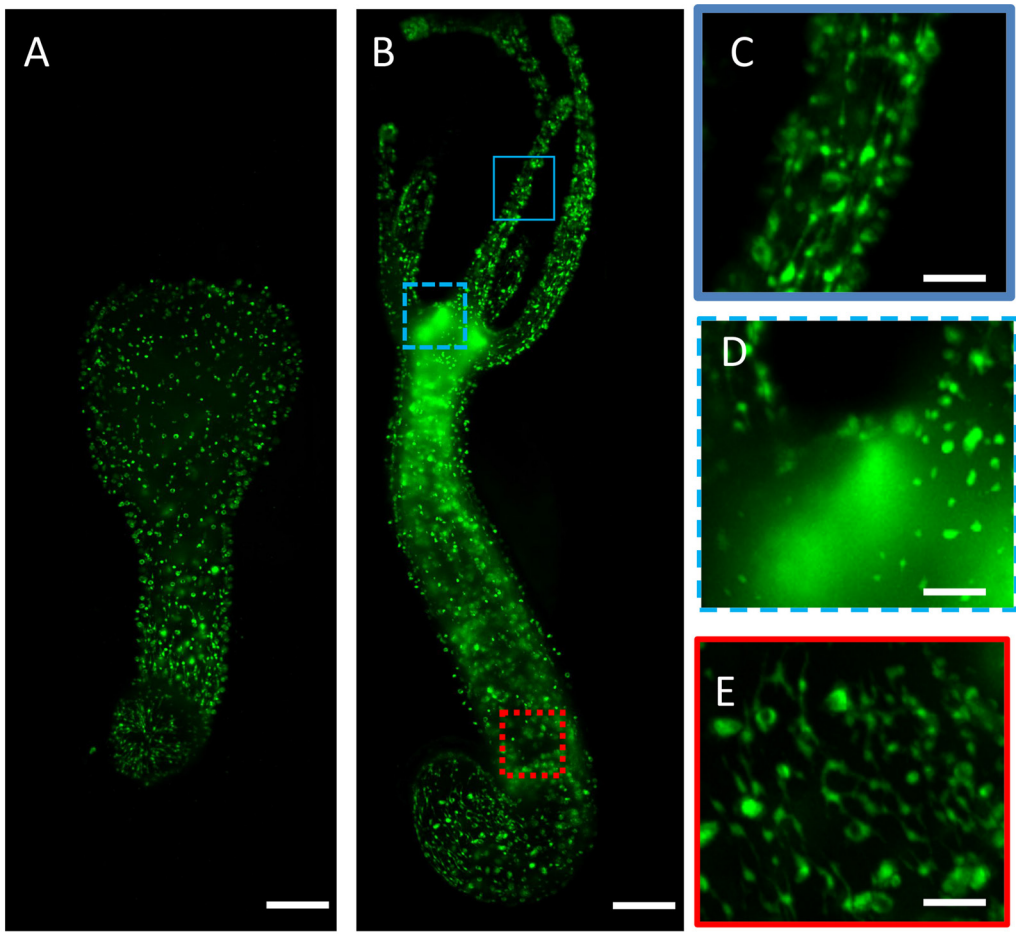


Fig. S11. Light sheet microscopy of a β-cat eGFP INT transgenic polyp. *Images were acquired immediately after amputation* **(A)** *and 96 h later* **(B)**. *Details of tentacles, hypostome and basal regions are showed in* **(C), (D),** *and* **(E).** *A clear up-regulation of eGFP reporter expression is detected in the head, progressively decreasing along the body gastric region.*

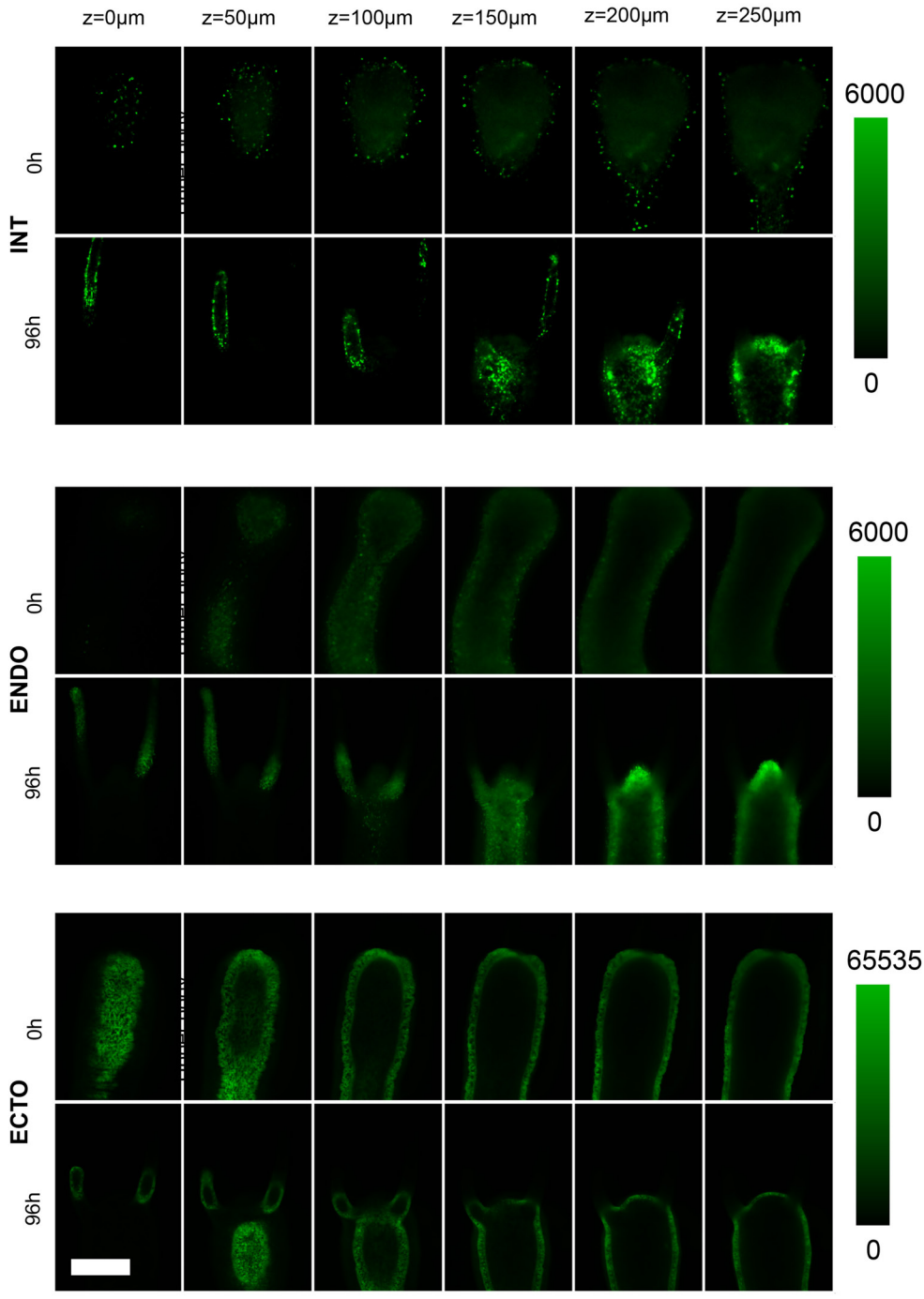


Fig. S12. Enhancement of eGFP expression in INT, ENDO and ECTO polyps during the regeneration process. *Single LSFM sections are shown every 50 µm (rows) at two time points, right after amputation (0 h) and at 96 h. Scale bar, 500 µm.*

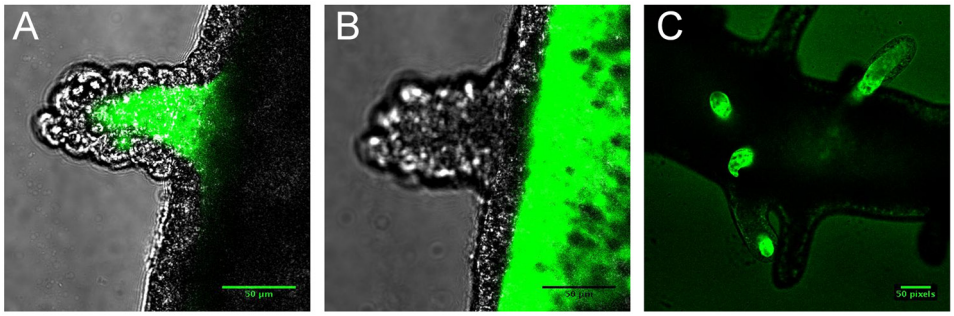


Fig. S13. Activity of β-cat promoter in β-cat -eGFP transgenic ENDO polyp. *Optical sections obtained by confocal microscopy at different animal depths* **(A)** *and* **(B)** *show participation of endodermal fluorescent cells to the formation of ectopic tentacles, showed at lower magnification in* **(C)**. *Scale bars 50 μm in A, B; 100 μm in C.*

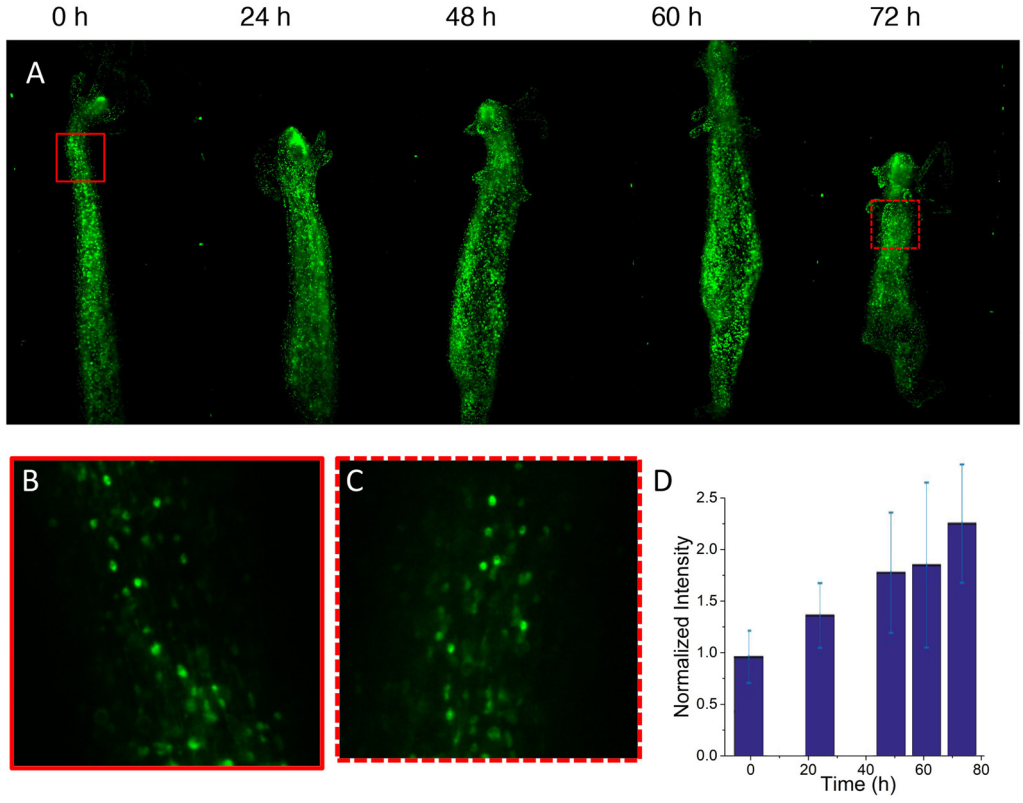


Fig. S14. Forced activation of Wnt signalling in β-cat-eGFP transgenic INT polyp. *β-cat-eGFP INT transgenic polyps were exposed 24 h to 5 μM ALP and then imaged with LSFM. Maximum Intensity Projections of the acquired stacks are shown every 24 h* **(A).** *Details of body regions (single LSFM sections) at the beginning* **(B)** *and at the end* **(C)** *of the acquisition are shown in the lower panels. The fluorescence intensity as a function of time, quantified on the body region of INT polyps (n=3) after segmentation of the LSFM data is shown in* **(D)**.

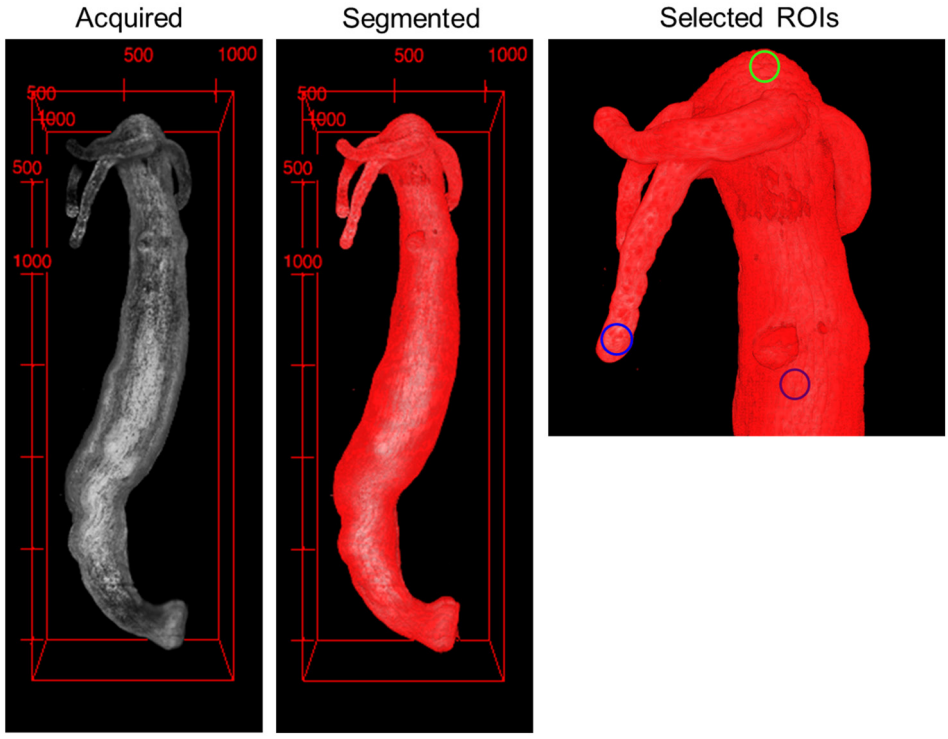


Fig. S15. Quantification of LSFM data. *The acquired stacks of images are automatically segmented using a threshold on the fluorescence intensity. The 3D segmented volume displayed by the Matlab software on the screen and the centre of the regions of interest (ROI) is chosen by the user (e.g. tentacle, hypostome upper body). The software computes the average fluorescence intensity on a 50X50X50μm volume: the sum of the intensity over the entire volume is calculated and divided by the total volume. The voxel outside the segmented volume are excluded from the calculation automatically. The volume is chosen at 10um from the external boundary of the segmented sample, closest to the detector.*